Eye (2003) 17, 943–948

www.nature.com/eye

New thoughts on the immunology of corneal transplantation

JW Streilein

Abstract

Significant advances derived from rodent models of penetrating keratoplasty have transformed our understanding of the pathogenesis of rejection of orthotopic corneal transplants. The high rate of success of corneal allografts placed in low-risk eyes without cover of immunosuppression arises from immune privilege of the cornea graft itself, and of the anterior chamber where it forms the anterior wall. Immune privilege owes its existence in penetrating keratoplasty to an absence of blood and lymph vessels in the graft and its bed, the absence of MHC class II$^+$ antigen presenting cells in the graft, reduced expression of MHC-encoded alloantigens on graft cells, constitutive expression of T cell-deleting CD95 ligand on corneal graft endothelium, the existence of an immunosuppressive local microenvironment (aqueous humor), and the capacity of the graft to induce anterior chamber associated immune deviation (ACAID). The results of recent experiments provide answers to pertinent questions regarding cornea graft failure: How does the cornea as a graft suppress inflammation and angiogenesis locally? How does the graft promote ACAID to the alloantigens it expresses? and How do corneal cells reduce their vulnerability as targets of effector T cells? The answers offer the possibilities of novel strategies for preventing immune-based corneal allograft failure.
Eye (2003) **17,** 943–948. doi:10.1038/sj.eye.6700615

Keywords: immune privilege; antigen presenting cells; major hisocompatibility complex; ACAID

Introduction

It has been more than 50 years since Rupert Billingham[1] and Sir Peter Medawar,[2] respectively, described the cornea as an immune privileged tissue and the anterior chamber as an immune privileged site. For the next 30 years, immune privilege of the anterior chamber was thought to result from 'immunologic ignorance'. Since this ocular site resides behind a blood : tissue barrier, and since it lacks a robust lymphatic drainage, these scientists reasoned that a foreign tissue graft was not rejected from the anterior chamber because the immune system could not detect the graft's presence. No credible explanations were advanced to explain the immune privileged status of the cornea. Subsequently, corneal surgeons took advantage of the cornea and eye's immune privileged status, and within a short period of time, penetrating keratoplasty became the most common and most successful solid organ transplantation in humans.[3]

Despite the relatively high rate of corneal graft acceptance in some patients (especially those considered to be 'low-risk'), other patients (termed 'high-risk') suffered cornea graft rejections at a frequency and intensity to rival that of heart and kidney grafts. It was clear that only research designed to unravel the pathogenesis of corneal graft failure would make it possible for strategies to be developed to improve the success of corneal transplants, especially in high-risk circumstances.

Experimental inquiry into the pathogenesis of corneal graft rejection changed dramatically in the late 1970s and early 1980s as a direct result of a significant technological advance. Williams and Koster[4] devised a surgical technique that made it possible to place cornea grafts orthotopically in eyes of rats. Shortly thereafter, the technique was adapted to permit orthotopic corneal transplants to be performed in mice. Owing to the power of inbred and genetically defined rodent strains combined with the specificity and sensitivity of reagents and assays enabling detailed study of rodent immune responses, a tremendous amount of new information has been generated. One purpose of

Schepens Eye Research Institute Harvard Medical School Boston, MA, USA

Correspondence:
JW Streilein
Schepens Eye Research Institute
Harvard Medical School
20 Staniford Street
Boston MA 02114, USA
Tel: +1 617 912 0100
E-mail: waynes@vision.harvard.edu

Received: 28 February 2003
Accepted in revised form: 28 February 2003

this communication is to review briefly some of the most important discoveries that have accrued during the past two decades, discoveries that have materially advanced our understanding of the physiologic bases of ocular immune privilege and of the pathogenesis of corneal allograft rejection and acceptance.

Lessons learned from two decades of corneal transplants in rodents

Some very important lessons have been learned from studying the fate of corneal transplants in rodents, and in analysing the immune responses animals make to these grafts.[5,6] Selected lessons of importance are enumerated below.

1. A significant proportion of allogeneic corneal transplants placed in the eyes of normal mice and rats neither experience immune rejection reactions nor undergo rejection. Since these experiments have been conducted without the aid of any local or systemic immunosuppression, this is *prima facie* evidence of the existence of immune privilege.

The high acceptance rate of orthotopic cornea grafts in normal eyes of untreated rodents correlates with the following:

(a) *Absence of blood and lymph vessels in the graft bed* (recipient rim): A very high percentage of corneal allografts placed in eyes experimentally manipulated to display inflammation and neovascularization are rapidly and vigorously rejected.

(b) *Absence of Langerhans cells in the graft epithelium*: Corneal allografts induced experimentally to contain Langerhans cells at the time of grafting rapidly sensitize their recipients, leading to their prompt rejection.

(c) *Reduced expression of major histocompatibility complex (MHC) molecules*: While MHC class I expression on corneal epithelial cells is strong, much less class I expression is detected on keratocytes, and even less on corneal endothelium. In the normal cornea, except at the limbal margins, no cells express MHC class II molecules. Since immune T cells can only detect potential target cells that express class I $\pm$ class II molecules, corneal cells, especially keratocytes and endothelial cells, represent rather poor targets for attacking donor-specific T cells.

(d) *Constitutive expression of CD95 ligand on corneal endothelial and epithelial cells*: Effector T cells, which carry the potential to destroy allografts, express CD95, a cell-surface molecule with a 'death domain'. If $CD95^+$ T cells encounter target cells that express the CD95 ligand, the T cells are triggered to undergo apoptosis, thus sparing the grafted target cell from rejection. This has been shown to be an important determinant of corneal allograft acceptance in mice.

(e) *Integrity of an immunosuppressive intraocular microenvironment*: If allogeneic corneas are placed in eyes in which immune privilege has been abolished, or in which aqueous humour lacks key immunosuppressive molecules, the grafts are rejected.

(f) *Induction of donor-specific anterior chamber-associated immune deviation (ACAID)*: Mice that accept orthotopic allogeneic cornea grafts indefinitely invariably lose the capacity to acquire and display donor-specific delayed hypersensitivity. Sustained acceptance of these grafts is believed to depend upon the persistence of this unresponsive state.

2. A variety of circumstances can prejudice against acceptance of orthotopic corneal allografts. Important factors that promote rejections include

(a) *Potent immunogenicity of graft epithelium*: When corneal allografts are placed at nonocular (heterotopic) sites, the epithelium has proven to be the most important component that promotes sensitization of the recipient to donor alloantigens. By contrast, stroma and corneal endothelium are (relatively or absolutely, respectively) unable to induce donor-specific sensitization.

(b) *Penetration of recipient antigen presenting cells (APCs), blood vessels, and inflammatory cells into the graft stroma*: In normal graft beds, angiogenesis that reaches the graft bears no relationship to whether the graft will be rejected or not. By contrast, inflamed and vascularized graft beds greatly enhance the rate at which new vessels and class II^+ Langerhans cells penetrate into the graft's stroma. Early sensitization to donor alloantigens and prompt rejection are the typical outcomes.

(c) *Emergence of donor minor H antigen-specific $CD4^+$ T cells of the 'indirect' type*: Whereas direct alloreactive T cells (specific for donor MHC antigens) play little or no role in promoting rejection of orthotopic corneal allografts, indirect alloreactive $CD4^+$ T cells (specific for donor allogeneic peptides presented on recipient class II MHC molecules) are solely responsible for rejection of orthotopic corneal allografts. Mice bearing allogeneic corneal allografts acquire donor-specific cytotoxic T cells, but these cells fail to contribute in any meaningful way to the rejection process. The ineffectiveness of $CD8^+$ T cells in rejection of orthotopic corneal allografts has yet to be explained.

Towards a molecular understanding of the recently learned lessons

Current experimental studies of corneal transplantation in rodents have the goal of expanding the knowledge base about the factors promoting acceptance and rejection, especially with respect to identifying the relevant molecular biology. The remainder of this communication presents selected recent experimental

results, some of which have yet to reach peer-reviewed publication. The purposes of these studies are to address the following important questions?

1. What strategies does the cornea use to suppress inflammation and angiogenesis within the graft, and within the graft bed?
2. How does the cornea promote immune tolerance (ACAID) of the alloantigens its cells express?
3. By what means do corneal endothelial cells lessen their vulnerability as targets of indirect alloreactive effector T cells?

Four separate experimental approaches to resolving these questions are described: (a) studies to identify a role for corneal epithelium in promoting survival of corneal allografts; (b) novel description of bone marrow-derived cells within the cornea stroma and epithelium; (c) molecular description of atypical expression of MHC class I and minor H antigens on corneal endothelium; and (d) molecular description of an atypical expression pathway for MHC class II molecules on corneal endothelium.

A role for corneal epithelium in promoting survival of corneal allografts

The corneal epithelium, as mentioned above, is believed to harbour strong properties of immunogenicity and as a consequence it is considered to be a potent inducer of recipient sensitization against alloantigens expressed by cornea grafts. In the rodent model system, the epithelium of long-accepted orthotopic corneal grafts has been shown to be rapidly replaced by recipient epithelium, raising the possibility that donor epithelium is largely irrelevant to cornea graft acceptance.[7] For this reason, Hori and Streilein[8,9] have examined whether corneal allografts deprived of the epithelial layer are less immunogenic than full-thickness grafts.

The results of an initial set of experiments indicated that, contrary to expectations, corneal allografts deficient in an intact epithelial layer are rejected much more rapidly than full-thickness grafts, and this correlated with the speed by which recipients of epithelium-deficient grafts became sensitized to donor alloantigens. In subsequent experiments, Hori and Streilein attempted to resurface epithelium-deprived corneal allografts with an epithelial layer genetically identical to the prospective recipient. This was accomplished by placing epithelium-deprived allogeneic (C57BL/6) cornea grafts in the eyes of immune incompetent mice (BALB/c SCID mice). These grafts were removed after 8 weeks (at which time the entire epithelium was of BALB/c genetic origin) and were then placed in the eyes of normal BALB/c mice. Surprisingly, these *in vivo* generated composite grafts sensitized their recipients exceedingly quickly, and the grafts were rapidly rejected. As a partial explanation of this unexpected result, it was demonstrated that *in vivo* reconstituted composite grafts of this type contained large numbers of MHC class II^+ Langerhans cells. The enhanced immunogenicity of these composite grafts was reasoned to result from the powerful capacity of Langerhans cells to process and present graft-derived alloantigens to recipient T cells.

To get around this problem, so-called alternative composite grafts were created *in vitro* by preparing from EDTA-treated corneas (a) sheets of pure BALB/c epithelium and (b) epithelium-deprived C57BL/6 corneas comprised of stroma and corneal endothelium. The epithelial sheets were floated on top of the stroma/endothelium *in vitro*, and then the composite grafts were sutured orthotopically into the eyes of BALB/c mice. As reported elsewhere, Hori and Streilein have found that composite grafts (BALB/c epithelium, C57BL/6 stroma and endothelium) were rarely rejected, whether placed in normal eyes of BALB/c recipients or in neovascularized eyes of the same strain. Moreover, mice bearing these composite grafts never acquired donor-specific delayed hypersensitivity, suggesting that the allogeneic stroma and endothelium of composite grafts are 'invisible' to the recipient immune system. This suggestion is strongly supported by the demonstration that composite graft survival can be promptly curtailed if mice bearing these grafts are cognately sensitized to donor alloantigens (a subcutaneous injection of C57BL/6 lymphoid cells).

This interesting set of results indicates that so long as the corneal epithelium is not itself expressing alloantigens with respect to the recipient, this surface tissue layer serves to suppress stromal inflammation and angiogenesis—both within the graft itself and within the graft bed. It is tempting to conclude that corneal epithelium possesses a unique capacity to promote cornea graft survival. Corneal epithelium might produce this salutary outcome by acting as (a) a barrier to proinflammatory agents from the tear film/ocular surface, (b) a source of anti-inflammatory and angiostatic factors (produced by the epithelium itself), and/or (c) a 'sink' that captures and inactivates proinflammatory and angiogenic factors released within the graft and its surrounding bed.

Bone marrow-derived cells within the cornea epithelium and stroma

The epithelium of normal corneas contains virtually no MHC class II^+ Langerhans cells. The absence of these cells has been linked causally to the immune privileged status of corneal allografts. In part this link derives from

the experimental observation that cornea grafts made experimentally to contain Langerhans cells within the epithelium are potent immunogens, rapidly sensitizing their recipients to donor alloantigens, and becoming the victims of vigorous rejection responses. From this line of investigation, a dogma emerged that the normal cornea is devoid of bone marrow-derived antigen presenting cells.

It was a surprise, therefore, when Liu *et al*[10] reported recently that cervical lymph nodes draining corneal allografts from transgenic green fluorescent protein (GFP)-expressing donors contained dendritic cells carrying the GFP marker. Moreover, the GFP^+ cells in these lymph nodes proved to be bone marrow-derived. The inescapable conclusion is that the normal cornea must contain mobile cells of bone marrow origin, and the prevailing dogma is incorrect. More recently, Hamrah *et al*[11] have formally documented that the epithelium of normal mouse corneas contains significant numbers of bone marrow-derived dendritic cells, even in the central region. However, these cells fail to express MHC class II molecules, accounting for the reason why cells of this type were not appreciated by previous investigators (who used class II expression as a reliable marker of the presence of these cells).

Pedram Hamrah and M Reza Dana (manuscript under review) have now studied whether the normal corneal stroma also contains MHC class II^- bone marrow-derived cells. These investigators have been rewarded by the discovery that three distinctly different populations of bone marrow-derived cells normally reside in the stroma. In the anterior stroma, a population of dendritic cells is deployed, cells that express the dendritic cell marker CD11c. In the posterior stroma, the predominant bone marrow-derived cell detected is $CD11b^+$, indicating the presence of macrophages. A similar finding was recently reported by Brissette-Storkus *et al*.[12] Finally, Hamrah and Dana have also detected a population of immature/progenitor dendritic cells distributed sparsely throughout the corneal stroma. Thus, it is necessary to reorder our thinking about bone marrow-derived cells and the normal cornea. While it remains true that MHC class II^+ Langerhans cells are not present, the normal corneal epithelium and stroma actually contain large numbers of MHC class II negative dendritic cells and macrophages. The biologic effects of these cells remain to be elucidated. One possibility is that corneal trauma or infection may release proinflammatory cytokines and factors that can activate these cells, permitting them to express class II molecules, and arming them to function as conventional antigen presenting cells. Another possibility is that resident class II^- dendritic cells and macrophages may be functionally programmed to promote tolerance of strong corneal antigens, of which alloantigens would be included. In this case, resident bone marrow-derived cells in the cornea may have the role of promoting corneal allograft acceptance.

Atypical expression of MHC class I and minor H antigens on corneal endothelium

Mice bearing orthotopic corneal allografts acquire donor-specific cytotoxic T cells. Yet, $CD8^+$ T cells appear to play *no* role in rejection of orthotopic corneal allografts. In addition, recent studies have demonstrated that minor histocompatibility antigens, rather than antigens encoded within the MHC, are the major barriers to cornea graft acceptance. Zdenka Haskova and Bruce Ksander (manuscript under review) have carried out a series of elegant studies designed to explain this curious set of findings. Using a well-defined murine model system in which cornea graft donor and recipient differ only with respect to a single minor histocompatibility antigen (H-3), these investigators first showed that corneal endothelial cells are highly resistant to lysis *in vitro* by specifically sensitized cytotoxic T cells. They then showed that corneal endothelial cells express very low levels of MHC class I molecules. Even when endothelial cells were treated with IFN-γ, which upregulated class I expression, lysis by cytotoxic T cells remained limited. Only when IFN-γ-treated corneal endothelial cells were pulsed with a critical H-3 antigen-derived derived peptide were corneal endothelial cells rendered vulnerable to cytotoxic T-cell lysis. The fact that provision of an exogenous peptide was required to convert endothelial cells into vulnerable targets suggests that these cells are unable on their own to generate similar peptides from endogenous proteins. Thus, corneal endothelial cells may be resistant to lysis by cytotoxic T cells because (a) they express very low levels of MHC class I molecules and (b) they lack proteosome-related enzymes that are required to generate the full range of immunogenic peptides to be loaded onto class I molecules. These authors have concluded that corneal endothelial cells avoid immune lysis because of a deficit in displaying antigenic peptides of endogenous proteins, and this may explain why $CD8^+$ T cells are largely irrelevant to corneal allograft rejection. In a larger sense, minor H antigens may be considered as surrogates for strong corneal endothelial autoantigens and viral-dependent antigens. Since corneal endothelium is largely incapable of replication, cytotoxic loss of endothelium is an irretrievable (and unacceptable) loss. It may well be that the unique pattern of MHC class I and minor H peptide expression displayed by corneal endothelial cells shields these cells from immune destruction, thus sparing vision.

Atypical expression pathway of MHC class II molecules on corneal endothelium

It has been an impenetrable conundrum of penetrating keratoplasty that tissue typing for MHC antigens is of little help in promoting cornea graft survival. Even clinical research centres that claim a positive influence of HLA matching on corneal graft outcome admit to a rather small effect. In addition, rejection of orthotopic corneal allografts in mice has been shown to depend largely on $CD4^+$ T cells of the so-called 'indirect' type. Indirect alloreactive T cells recognize allogeneic peptides of donor origin when displayed on self-MHC class I and II molecules. 'Direct' alloreactive T cells, in contrast, are capable of recognizing donor MHC alloantigens directly, irrespective of peptides associated with these molecules. We have wondered whether there is a molecular explanation that could link the relative lack of MHC matching on graft outcome with the primacy of indirect alloreactive $CD4^+$ T cells as effectors of graft rejection. Since $CD4^+$ T cells use MHC class II molecules as restricting elements, Carolina Arancibia-Carcamo (manuscript under review) has studied the details of MHC class II expression by murine corneal endothelial cells.

Whether transformed corneal endothelial cells are studied or intact corneal endothelium of an anterior eyecup is examined, corneal endothelial cells never express MHC class II molecules in the unmanipulated state nor do these cells express class II molecules when exposed to IFN-γ alone, or TNF-α alone, cytokines that typically induce class II expression on most other cell types. Only when corneal endothelial cells are exposed simultaneously to both TNF-α and IFN-γ do they express class II molecules. When they do so, however, they express neither CIITA nor invariant chain, indicating the the conventional activation pathway via the IFN-γ receptor is not involved. In fact, no evidence of transcription of either of these genes has been detected in normal or cytokine-treated corneal endothelial cells. Thus, treatment of corneal endothelium with proinflammatory cytokines has the unexpected effect of triggering CIITA-independent expression of class II molecules. The biologic meaning of this curious outcome is suggested when cytokine-treated corneal endothelial cells are transfected to express the heterologous antigen ovalbumin in the cytosol or within endosomal vesicles. Only in the former instance are relevant ovalbumin peptides presented on the surface of corneal endothelial cells, rendering the cells capable of activating OVA-specific $CD4^+$ T cells *in vitro*. This indicates that class II-expressing corneal endothelial cells preferentially display endogenous, rather than exogenous, peptides to $CD4^+$ T cells. We believe that this largely explains why corneal endothelium can serve as the target of $CD4^+$ T cells in corneal allograft rejection. Moreover, this mechanism explains why minor H antigens are such important targets of these T cells. By definition, minor H antigens are endogenous proteins, and they provide peptides to be loaded onto class II molecules expressed in the absence of invariant chain.

We interpret these findings to mean that when exposed to inflammatory stress, corneal endothelial cells express class II in a manner that displays endogenous peptides (minor H antigens), and this accounts for the special vulnerability of corneal allografts to $CD4^+$ T-cell immunity directed at minor H antigens. However, we are aware of the fact that both normal and class II-expressing corneal endothelial cells lack the expression of costimulation molecules for T-cell activation. This deficiency raises the possibility that T cells that encounter class II-expressing corneal endothelial cells might be anergized or even tolerized by this experience, thus protecting the cornea from the possibility of a T-cell-motivated immune attack.

General conclusions and implications

Laboratory research is beginning to find molecular explanations for several manifestations of immune privilege that correlate with the high rate of acceptance of corneal allografts in low-risk eyes. The hope is that molecular understanding of this type will lead eventually to novel treatment and prevention strategies designed to promote cornea graft acceptance, even in high-risk eyes. Current lines of investigation indicate that the corneal epithelium provides physical and molecular shields that suppress inflammation and angiogenesis within the graft stroma and the graft bed. Class II^- dendritic cells and macrophages are present in the normal cornea stroma and epithelium, raising the probability that cells within the cornea can address the recipient immune system directly—for good or evil. Corneal endothelial cells have distinct molecular strategies to reduce their antigenic visibility to $CD4^+$ and $CD8^+$ effector T cells, and to alter the functional program of responding T cells.

It is altogether possible that these (and other) newly discovered features of the cornea may prove to be important in promoting the acquisition of immunologic tolerance directed at cornea-derived antigens. It is axiomatic that high-quality vision depends upon the unique physiologic features of the corneal stroma, maintained by the equally important actions of corneal endothelium. Since replication of corneal endothelial cells and restoration of a disordered corneal stroma are difficult to accomplish once damage to the cornea has been sustained, deterioration of vision is a considerable

threat to quality of life. The ability of the cornea to promote immunologic unresponsiveness may be an evolutionary adaptation designed to preserve vision—at all costs—by avoiding immunogenic inflammation. Immune privilege is one way of expressing this adaptive, sight-sparing strategy.

Acknowledgements

I greatly appreciate the willingness of Drs Pedram Hamrah, M Reza Dana, Zdenka Haskova, Bruce Ksander, and Carolina Arancibia-Carcamo to permit me to present some of their important and seminal recent experimental findings.

References

1 Billingham RE, Boswell T. Studies on the problem of corneal homografts. *Proc R Soc Lond B* 1953; **141**: 293–406.
2 Medawar P. Immunity to homologous grafted skin. III. The fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *Br J Exp Pathol* 1948; **29**: 58–69.
3 Brady SE, Rapuano CJ, Arentsen JJ, Cohen EJ, Laibson PR. Clinical indications for and procedures associated with penetrating keratoplasty. *Am J Ophthalmol* 1989; **108**: 983–988.
4 Williams KA, Coster DJ. Penetrating corneal transplantation in the inbred rat: a new model. *Invest Ophthalmol Vis Sci* 1985; **26**: 23–30.
5 Streilein JW. Immunobiology and immunopathology of corneal transplantation. In: Streilein JW (ed). *Immune Response and the Eye*. Karger, Basel, Switzerland, 1999, pp 186–207.
6 Niederkorn JY. The immune privilege of corneal allografts. *Transplantation* 1999; **67**: 1503–1508.
7 Hori J, Joyce N, Streilein JW. Where immune privilege and immunogenicity reside among different layers of the mouse cornea. *Invest Ophthalmol Vis Sci* 2000; **41**: 3032–3042.
8 Hori J, Streilein JW. Role of recipient epithelium in promoting survival of orthotopic corneal allografts in mice. *Invest Ophthalmol Vis Sci* 2001; **42**: 720–726.
9 Hori J, Streilein JW. Survival in high risk eyes of epithelium-deprived orthotopic corneal allografts reconstituted *in vitro* with syngeneic epithelium. *Invest Ophthalmol Vis Sci* 2003; **44**: 658–664.
10 Liu Y, Hamrah P, Zhang O, Taylor AW, Dana MR. Draining lymph nodes of corneal transplant hosts exhibit evidence for donor major histocompatiblity complex (MHC) class II-positive dendritic cells derived from M<HC class II-negative grafts. *J Exp Med* 2002; **195**: 125–129.
11 Hamrah P, Zhang O, Liu Y, Dana MR. Novel characterization of MHC class II-negative population of resident corneal Langerhans cell-type dendritic cells. *Invest Ophthalmol Vis Sci* 2002; **43**: 639–646.
12 Brissette-Storkus CS, Reynolds SM, Lepisto AJ, Hendricks RL. Identification of a novel macrophage population in the normal mouse corneal stroma. *Invest Ophthalmol Vis Sci* 2002; **43**: 2264–2271.